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(54) Title: MONOCOT SEED GENE EXPRESSION SYSTEM

(57) Abstract

A gene construct comprising the rice glutelin-1 promoter operably connected to a foreign non-plant gene is disclosed. A monocot seed containing a gene construct comprising a rice glutelin-1 promoter operably connected to a foreign non-plant gene is also disclosed. Preferably, the gene product is expressed at a level of at least 1 % of total soluble endosperm protein. Most preferably, the gene product is expressed at a level of at least 2.5 % of the total soluble endosperm protein.

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MONOCOT SEED GENE EXPRESSION SYSTEM

Field of the Invention

The field of the present invention is plant transformation systems. In particular, the field of the present invention is a system for expressing foreign genes in maize kernels by use of the rice glutelin-1 promoter.

Background

Plant Transformation

One general object of modern biotechnology is to genetically engineer crop plants by introducing new genetic traits into the genome of elite plant lines. Plants with new traits, such as insect resistance or herbicide resistance, and artificial manipulations of the agronomic qualities of the crop product are possible once recombinant genes are introduced into plant lines.

The first widely used plant genetic engineering technique was based on the natural ability of the soil-dwelling microorganism Agrobacterium tumefaciens to introduce a portion of its DNA into a plant cell as a part of the normal pathogenic process. If a foreign gene is inserted into the bacteria in certain ways, the Agrobacterium can be used to transfer the foreign gene into a plant. Agrobacterium transformation techniques have b en developed for a number of plants, mostly dicotyledonous, but the usefulness of the technique has

varied from plant species to species. Agrobacterium-based transformation systems are limit d because they require cell or tissue culture and plant regeneration techniques. Plants lines vary in their amenability to tissue culture and regenerations methods. Regeneration of maize protoplasts has been accomplished recently, but the efficiency of the process is low. Rhodes, et al., Bio/technology 6:56-60 (1988). A maize genetic engineering system which obviates tissue culture and regeneration would represent a significant advantage.

One new developing technique for creating transformed plants includes bombarding a cell with accelerated particles carrying genetic information. The first indication of the utility of this technique was a demonstration that DNA constructs could be coated onto tungsten particles and accelerated into onion skin where the genes were transiently expressed, as is described in the specification of U.S. Patent No. 4,945,050. A problem in the development of an accelerated particle transformation process to create transgenic plants is the difficulty of obtaining a germline plant transformation. By the term "germline transformation" we mean that the germ cells of the plant are transformed in such a way that the progeny of the plant inherit the inserted foreign genetic construct. In some species, plant genetic transformation has been achieved by the accelerated particle method. European Patent Application No. 301,749 discloses the germline transformation of soybean plants and plant lines. The method disclosed in that published patent application is based on accelerating DNA-coated particles into the excised embryonic axes of soybean seeds. If the bombarded soybean embryonic axes are treated with high cytokinin media, shoots are induced from the treated embryonic axes. When the shoots are cultivated into whole soybean plants, a significant percentage of the plants will have transformed germlines.

Accelerated particle transformation has also been successful in treatment of maize suspension cultures. Fromm, et al., Bio/Technology 8:833-839 (1990), discloses the creation of transformed maize plants from suspension cultures and from calli created from suspension cultures. Klein, et al., Proc. Natl. Acad. Sci. USA 85:4305-4309 (1988), discloses accelerated particle transformation of maize suspension culture cells. Spencer, et al., Theor. Appl. Genet. 79:625-631 (1990), discloses stable transformed maize callus recovered from suspension culture cells bombarded with DNA-coated accelerated particles. Gordon-Kamm, et al., The Plant Cell 2:603-618 (1990), discloses microprojectile bombardment of embryogenic maize suspension cultures. None of these disclosures deal with transformation of tissues not in cell culture. Most elite lines of maize cannot presently be regenerated from suspension cell cultures. Hence, prior techniques may be limited to certain less desirable genotypes of maize plants.

Maize embryos have been bombarded and transient expression achieved by DNA-coated particles. Klein, et al., Bio/Technology 6:559-563 (1988), discloses particle bombardment of surface cells of an excised maize embryo. This reference does not disclose regeneration from the bombarded embryo, nor inheritance in progeny plants. The cells were bombarded with DNA encoding the beta-glucuronidase (GUS) gene. Because it turns a substrate, 5-bromo-4-chloro-3-indolyl glucuronidide, blue in an in situ tissue assay, beta-glucuronidide, can be histochemically detected. After bombardment and treatment with the GUS substrate, blue spots appeared on the embryo, indicating transiently transformed cells.

Koziel, et al., Biol. Technology 11:194-199, 1993, describe the introduction of a synthetic gene encoding a truncated version of the CryIA(b) protein derived from Bacillus thuringiensis into immature embryos of

maize using microprojectile bombardment.

Rice Glutelin-1 Promoter

One recently identified plant promoter has been identified as driving the transcription of the rice glutelin-1 gene. The rice glutelin-1 promoter has been described in Zheng, Z., et al., The Plant Journal 4:357-366 (1993) and Okita, T.W., et al., The J. Biol. Chem. 264:12573-12581 (1989). Zheng, et al. (Plant Physiol. 109:777-786, 1995) disclose the attachment of either a 5.1 or 1.8 kb promoter fragment of the rice seed storage protein glutelin GT-1 gene to either the β-phaseolin genomic or cDNA sequence. The β-phaseolin protein was successfully synthesized, processed and accumulated in rice endosperm.

What is needed in the art of molecular biology is a maize kernel gene expression system by which gene products can be highly expressed in a maize kernel. Maize kernels containing high levels of specific heterologous gene products will be very useful for modifying seed composition, altering protein nutritional composition and facilitating purification of specific heterologous proteins from maize kernels.

Summary of the Invention

In one embodiment, the present invention is a monocot seed, plant, or cell containing a gene construct comprising the rice glutelin-1 promoter operably connected to a foreign non-plant gene, wherein the promoter and the gene are not natively connected. Preferably, the gene is a mammalian gene and, preferably, the seed, plant or cell is a maize seed, plant or cell.

In another embodiment of the present invention, the product encoded by the foreign gene is expressed at a level of at least 1% of the total soluble endosperm protein. More preferably, the product is expressed at a level of at least 2.5% of the total soluble endosperm

protein. Most preferably, the product is at least 5% of the total soluble endosperm protein.

The present invention is also a gene construct comprising the rice glutelin-1 promoter operably connected to a gene encoding a non-plant protein, preferably a protein such as an antibody.

In one embodiment of the invention, the rice glutelin-1 promoter is the 4888 bp fragment described in SEQ ID NO:1. In another embodiment of the present invention, the rice glutelin-1 promoter is a 1.8 kb fragment of the 4888 bp promoter. This 1867 bp fragment extends from residue 3021 to residue 4888 of SEQ ID NO:1.

In another embodiment of the present invention, the present invention is method of creating a transformed monocot plant, seed, or cell. This method comprises the steps of creating the gene construct described above, creating a transgenic plant comprising the gene construct, and allowing expression of the non-plant gene.

It is an advantage of the present invention that the rice glutelin-1 promoter operably connected with a foreign non-plant gene provides an expression system in maize kernel that results in the foreign gene expression product comprising a significant amount of the total soluble protein. This abundance of foreign protein will facilitate purification of the product and may also improve the nutritional quality of seed protein.

It is a further advantage of the present invention that the foreign gene product may be an antibody protein, thus allowing one to more easily produce or isolate recombinant antibodies.

It is a further advantage of the present invention that a gene product of agricultural importance may be expressed in the maize kernel. Thus, one may have increased expression of a gene product that would add nutritive value or beneficial properties to the maize

product itself.

Other objects, features and advantages of the present invention will become apparent to one skilled in the art after examination of the specification, drawings and claims.

Description of the Drawings

Fig. 1 is an exploded schematic view of a particle acceleration device useful in the present invention.

Fig. 2 is a top plan view of the device of Fig. 1.

Fig. 3 is an enlarged drawing of the corn immature embryo stage 1.

Fig. 4 is a schematic diagram of plasmid WRG5084.

Fig. 5 is a schematic diagram of plasmid WRG5086.

Fig. 6 is a schematic diagram of plasmid WRG4564.

Fig. 7 is a schematic diagram of plasmid WRG5236.

Fig. 8 is a schematic diagram of plasmid WRG5243.

Fig. 9 is a schematic diagram of plasmid WRG5245.

Description of the Invention

1. In General

This invention relates to the economical production of heterologous proteins in monocotyledonous (monocot) plants such as maize. Preferably the heterologous proteins are pharmaceutical proteins such as antibodies. The proteins are preferably expressed in monocot seeds such as maize kernels. More specifically, the proteins are preferably expressed in the maize seed endosperm. High levels of expression of heterologous proteins, especially of monoclonal antibodies, have been difficult to achieve in monocots before, partly because of the lack of suitable seed-specific promoters.

In one embodiment, the present invention is a monocot seed, such as a maize kernel, containing a gene construct comprising the rice glutelin-1 promoter operably connected to a foreign non-plant gene. The gene product encoded by the foreign gene is preferably

expressed at a level of at least 1% of the total soluble endosperm protein and most preferably is expressed at a level of at least 5% of the total soluble protein in seed or kernel endosperm.

We report below in the Examples the high levels of expression of monoclonal antibody BR96 in maize endosperm when the heavy chain and light chain genes are driven by the rice glutelin-1 promoter. Additionally, we have described the successful production of a second monoclonal antibody, NeoRx451.

2. Suitable Promoters and Preparation of Nucleic Acid Constructs

The present invention requires a gene construct in which the rice glutelin-1 promoter is operably connected to a foreign gene. The Examples below disclose one particularly useful embodiment of the rice glutelin-1 promoter that was obtained from Dr. N. Murai (Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana 70803-1720) as part of clone pGt5.1(BglII). To obtain the promoter, a HindIII site was inserted into the BglII site destroying the BglII site and resulting in pWRG4563. The rice glutelin-1 promoter was removed from pWRG4563 as an approximately 4.9 kb Kpn1-HindIII fragment and inserted into the Kpn1-HindIII site of pWRG2484 to make pWRG4564 (glutelin promoter and leader-amv leader-GUS). Fig. 6 is a map of pWRG4564.

The rice glutelin-1 promoter was then moved as a XhoI-HindIII fragment into both pWRG2800 and pWRG2801 to make pWRG4565 (glutelin promoter and leader-tobacco extensin leader and signal peptide-BR96 heavy chain) and pWRG4566 (Glutelin promoter and leader-tobacco extensin leader and signal peptide-BR96 light chain respectively), respectively. The tobacco extensin fragment contains all of the 5' UTR and the first 26 amino acids of ext nsin coding region (De Loose, et al., Gene 99:95-100, 1991). 35S-hygromycin resistance cassettes were inserted into both of these plasmids

resulting in pWRG5086 and pWRG5084 (Figs. 4 and 5).

The nucleotide s quence of the Kpn1-HindIII fragment from pWRG4563 was determined and is listed at SEQ ID NO:1. Sequencing of both strands was performed by Retrogen Inc. using Applied Biosystems automated sequencer. The transcription initiation site as described by Okita, et al., is at residue 4861 of SEQ ID NO:1. Nucleotides -4861 to +27 of the rice glutelin-1 gene (as numbered by Zheng, et al. The Plant Journal 4:357-366 (1993)) correspond to nucleotides 1-4888 in SEQ ID NO:1. One may use the nucleotide sequence reported in SEQ ID NO:1 to design primers for direct PCR amplification of the rice glutelin-1 promoter from a rice genomic library.

One may also obtain the rice glutelin promoter by following the method described by Okita, et al., The J. Biol. Chem. 264:12573-12581 (1989).

Although the full-length (4888 bp) rice glutelin promoter was used in the experiments below, Applicants envision that a smaller fragment would also be suitable, although it is believed that the 4888 bp fragment is preferable. A 1867 bp fragment of the 4888 bp promoter has been demonstrated to promote gene expression, although with less strength than the larger fragment. This 1867 bp fragment extends from the EcoRI site to the BglII site of pGT5.1 as described in Zheng, et al., The Plant Journal 4:357-366 (1993). This fragment corresponds to residues 3021 to 4888 of SEQ ID NO: 1. Therefore, a suitable glutelin-1 promoter will contain at least this 1867 bp sequence. Expression levels may be further increased by adding some intron sequences.

One of skill in the art of molecular biology will also realize that minor additions and subtractions to a promoter sequence can result in a promoter with nearly identical activity to the wild type promoter. These manipulated or mutated sequences would be just as suitable and are considered "rice glutelin-1

promoters."

To determine whether a fragment or mutation is suitable for the present invention, one would assay it side-by-side the rice glutelin-1 promoter described above and measure gene expression in a system utilizing a plasmid such as described at Figs. 4 and 5. Any gene expression of at least 75% that displayed by the native rice glutelin-1 promoter indicates that the promoter fragment or modification is suitable.

A preferable gene construct will comprise at least a rice glutelin-1 promoter operably connected to a foreign non-plant gene. However, other expression elements are useful in various embodiments of the gene construct of the present invention. In particular, signal peptide sequences are useful for protein products that are expected to be secreted through membranes. For example, the antibodies described below in the Examples were connected to the tobacco extensin signal peptide for proper membrane processing.

Additionally, a portion of the rice glutelin untranslated region sequence (UTR) is a preferable addition.

The Examples below describe gene constructs that are preferable for expressing antibodies in the method of the present invention. Elements in the constructs used below in the Examples and diagramed at Figs. 4 and 5 are described in the following sources: Adh intron: Callis, J., et al., (1987) Genes Dev. 1:1183-1200; GUS gene: Jefferson, R.A., et al., (1987) EMBO J. 6:3901-3907; tobacco extensin gene: De Loose, M., et al., (1991) Gene 99:95-100; hygromycin: Van den Elzen, P.J.M., et al., (1985) Plant Mol. Biol. 5:299-302; and vector backbone: Yanisch-Perron, C., et al. (1985) Gene 33:103-119.

As described above, foreign genetic constructions are often embodied in expression cassette vectors for plant cells, many of which are known in the art. These vectors may be part of a plasmid or a virus. Typically

such a plant expression vector system includes the coding sequence for the desired foreign gene and appropriate regulatory sequences. The appropriate regulatory sequences might include a translational terminator and signal or leader sequences. A translation or transcriptional enhancer may be incorporated between the promoter and the coding region of the genetic sequence. One with knowledge in the art of molecular biology would know how to use standard techniques to insert the rice glutelin-1 promoter upstream, or 5', of the coding region of any particular gene.

The transforming nucleic acid construct can include a marker gene which can provide selection or screening capability in the treated plant tissues. Selectable markers are generally preferred for plant transformation events, but are not available for all plant species. A selectable marker encodes for a trait in the transformed plant cells which can be selected for by the exposure of the plant tissues to a selection agent. Suitable selectable markers can be antibiotic or herbicide resistant genes which, when inserted in some cells of a plant in culture, would imbue those particular cells with the ability to withstand exposure to the antibiotic or the herbicide.

It has been found that at least one antibiotic selectable marker system can be made to work in maize. A gene coding for resistance to the antibiotic hygromycin from E. coli has previously been found to be useful as a selectable marker in tobacco transformation. Waldron, et al., Plant Mol. Biol. 5:103-108 (1985). Such selectable markers do not reliably transfer from species to species, particularly when used on callus or differentiated tissue as opposed to protoplasts or suspension culture.

Another type of marker gene is one that can be screened by histochemical or biochemical assay, even though the gene cannot be selected for. A suitable

marker gene found useful in such plant transformation experience is the GUS gene, discussed above. Jefferson, et al., EMBO J. 6:3901-3907 (1987) discloses the general protocol for a GUS assay. Thus, the use of a GUS gene provides a convenient colorimetric assay for the expression of introduced DNA in plant tissues by histochemical analysis of the plant tissues. In a typical transformation process, the gene of interest sought to be expressed in the plant can be coupled in tandem with the GUS gene. Then the tandem construct can be transformed into plant tissues and the resulting plant tissues can be analyzed for expression of the GUS enzyme. Tissues and plants expressing the GUS enzyme will also be found to be expressing the gene of interest.

3. Suitable Foreign Genes

In one embodiment, the present invention is a monocot seed, such as a maize kernel, containing a gene construct comprising the rice glutelin-1 promoter operably connected to a foreign non-plant gene. By "foreign non-plant gene" we mean a gene that is not natively connected to the rice glutelin-1 promoter and is not isolated from plant sources. Preferably, the gene is of mammalian origins or is synthetically created. A "foreign non-plant gene" may also be a synthetic gene that is created to mimic a non-plant gene. Specifically, a "mammalian gene" may include a synthetic gene designed to mimic a mammalian protein such as an antibody. A "non-plant gene" or a "mammalian gene" may be a chimeric gene.

Preferably, the foreign gene encodes an antibody or a fragment of an antibody. The Examples below demonstrate the successful production of both the heavy and light chains of monoclonal antibody BR96 and antibody NeoRx451.

4. Preparation of Monocot Embryos

We envision that the present invention will be useful in creating a variety of transgenic

monocotyledonous plants. Most preferably, the invention involves the creation of a transgenic maize plant, seed or kernel. Below, we describe a preferred method of transformation of maize embryos. One of skill in the art will realize that these methods may be adapted to work in other monocotyledonous plants such as rice and wheat.

A gene construct including the rice glutelin-1 promoter will be effective at enhanced gene expression in a variety of transformation systems and methods. Below in the next three sections we list a preferred method of preparing maize embryos, transforming the embryos and regenerating the plants. One of skill in the art of molecular biology would realize that variations of this transformation procedure would also be effective.

Maize kernels are sterilized and isolated from conditioned ears. Preferably, the ears from which these kernels are taken have been conditioned at 4°C from one to eight days with two to four days being preferred. The maize embryo, which is composed of the scutellum and the shoot/root axis, is then excised from the kernel. The scutellum is the cotyledon of the maize embryo. Esau, Anatomy of Seed Plants, John Wiley & Sons, New York, p. 477 (1960).

Preferably, immature embryos are excised. By "immature" we mean that the embryos are approximately between 0.50 mm and 2.50 mm in length are in the developmental stages of early coleoptilar, stage one or stage two, as defined by Abbe and Stein Am. Jour. Bot. 41:285-293 (1954). The embryos just entering stage one are preferred. Mature embryos may be used in the present invention, but it is more difficult to culture these embryos. Very immature embryos may be used, but these smaller mbryos are harder to manipulate.

The maize embryos are placed on a medium amenable to immature embryo scutella culturing. The embryonic axis should touch the media, and the scutellum should

be exposed. Preferably, the medium should contain the salts and carbon source (sucrose or other sugars) needed for growth, an auxin or auxin-like substance, and agar or other gelling agent. In the practice to date, the culturing medium contained chloramben, an auxin. Other auxins, such as 2,4-D may also be used. The embryos are incubated in the dark for conditioning. Usually, this takes about four days.

Preferably, the callus that develops on the scutellum is a mixture of organogenic and embryogenic callus. Almost every maize line is susceptible to this type of callus culture. For example, McCain and Hodges, Bot. Gaz. 147[4]:453-460 (1986), disclose a method of obtaining somatic embryos that have developed from scutellum of immature zygotic embryos of maize variety A188.

In transformation efforts using Agrobacterium-based procedures with other crops, was found that wounded tissue often gave a better transformation result. This phenomenon has not been demonstrated to be useful in accelerated particle transformation methods. Nevertheless, in the protocol used here a part of the embryo that is not producing callus is preferably excised to optimize transformation results. The non-producing tissue is visually identified by the lack of visible callus proliferation. Typically, the callus proliferates mainly at the suspensor end of the immature embryo on abaxial side of scutellum. The portion of the embryo that is removed is from the coleoptile end. This is illustrated in Fig. 3, illustrating a stage 1 embryo modelled after Abbe and Stein, Am. Jour. Bot. 41:287 (1954). The suspensor end 2 is where the callus proliferates on the lower one-half to three-quarters of the scutellum. The top one-half to one-quarter of the coleoptile end 3 of the scutellum is the part that is removed. A dashed line 4 indicates the usual locus of the art. The removed scutellum portion is discarded.

The dissected embryos are placed on fresh medium and positioned so that the callus tissue is available to be bombarded by the accelerated particles. Preferably, this medium now contains an osmoticum such as 0.4 M mannitol, so that the embryogenic cells will be partly plasmolyzed. This osmotic condition helps to preserve cell integrity through bombardment. Optimally, the embryos should remain on this medium one to three hours at room temperature in the dark before they are bombarded.

5. Transformation of Embryos

A. Preparation of Nucleic Acid-Coated Particles

Multiple copies of the nucleic acid construct are prepared by known molecular biology techniques.

The transformation process requires carrier particles of a durable, dense, biologically-inert material. Gold is a suitable material for use as the carrier particle. The carrier particles are of extremely small size, typically in a range of 0.7 to 3 microns, so that they are small in relation to the size of the maize target cells. Preferably, microcrystalline gold particles are used as carrier particles. A suitable source of microcrystalline gold particles is Degussa/Metz (Lot #7-29020-0, Gold Beads 0.71 μ). This product consists of gold particles of high surface area and amorphous shape and size. We found that microcrystalline carrier particles of irregular size achieve a higher transformation efficiency than that achieved by spherical gold particles.

The genetic material to be inserted into the cells is coated onto the carrier particles. Both heavy chain and light chain plasmids are coated onto the gold beads simultaneously for co-transformation. The DNA loading ratio for each plasmid was 2 μg/mg of gold particles. This can be readily done by drying solutions of DNA or RNA onto the carrier particles themselves. Suitable stabilizers can be added to the mixture to help with

the longevity of the genetic material on the carrier particles, such as the preparation based on spermidine described in the Example below.

B. Bombarding Cultured Embryos

The apparatus utilized in the present invention must be capable of delivering the nucleic acid-coated particles into plant cells in such a fashion that a suitable number of cells can be transformed. At some frequency, the carrier particles lodge within the maize cells and, through a process we do not understand, the genetic materials leaves the carrier particles and integrates into the DNA of the host maize cells. Many types of mechanical systems can accelerate the carrier particles into plant cells. Possible mechanisms include ballistic explosive acceleration of particles, centrifugal acceleration of particles, electrostatic acceleration of particles, or other analogous systems capable of providing momentum and velocity to small particles.

The mechanism we used in the Example is based on the acceleration of particles through an adjustable electric voltage spark discharge device which is capable of accelerating a planar carrier sheet at a target surface. This apparatus will be described further below with reference to Figs. 1 and 2.

The particle acceleration apparatus is generally indicated at 10 of Fig. 1. The apparatus consists of the spark discharge chamber 12 into which are inserted two electrodes 14 spaced apart by a distance of approximately one to two millimeters. The spark discharge chamber 12 is a horizontally extended rectangle having two openings, 16 and 18, extending out its upward end. The opening 16 is covered by an access plate 20. The opening 18, located on the side of the rectangle of the spark discharge chamber opposite from the electrode 14, is ultimately intended to be covered by a carrier sheet 22.

The electrodes 14 are connected to a suitable

adjustable source of electric discharge voltage (not shown). A suitable source of electric discharge voltage includes a capacitor in the size range of one to two microfarad. The voltage of the charge introduced into the capacitor should be adjustable. An adjustable voltage can be introduced readily into such a capacitor through the use of an autotransformer which can be adjustable between a range of one and fifty thousand volts. Preferably, a high voltage electric switch is provided so that the capacitor can be discharged safely through the electrodes 14 without harm to the operator.

A carrier sheet 22 is placed upon the opening 18 of the spark discharge chamber 12. The carrier sheet 22 is a planar sheet of relatively stiff material which is capable of carrying small, inert carrier particles thereon toward the target surface. Preferably, the carrier sheet 22 is a small sheet of aluminized, saran-coated mylar. We envision that other relatively stiff, planer materials may be used for the carrier sheet 22. The function of the carrier sheet 22 is to convert an outwardly outstanding force produced by the electrodes to a broadly distributed horizontal force capable of accelerating a large number of carrier particles in parallel with an even force. Other kinds of force other than electric discharge can be used to propel the carrier sheet 22 upward. The force should be adjustable so that the force of travel of the carrier sheet 22 can be adjusted.

Again referring to the apparatus of Figs. 1 and 2, a retaining screen 24 is approximately 15 millimeters above the opening 18 and the discharge chamber 12. A target surface 26 is placed above the retaining screen 24 at a distance of between 5 and 25 millimeters. The target surface 26 is any suitable culture surface onto which the material to be transformed can readily be placed. An overturned petri dish can conveniently be used for the transformation of plant tissues. Using a

semisolid or solid agar-based medium in the bottom of a petri dish, it is possible to place tissues on the agar where they will be retained. The petri dish itself can serve as the target surface while retaining the plant tissues on the agar.

The DNA-coated particles are layered onto the top of the carrier sheet 22. The layering is done so as to distribute a relatively even pattern of carrier particles across the entire top surface of the carrier sheet 22. Preferably, the coated carrier particles are applied to the carrier sheet at a loading rate of .025 to .050 milligrams of coated carrier particles per square centimeter of carrier sheet. The carrier sheet 22 is placed upon opening 18. An oil or water droplet is used to adhere the carrier sheet 22 in place. The target surface 26, with the living plant material thereon, is placed in position above the retaining screen 24. A small droplet of water, preferably 10 microliters, is placed in the chamber bridging between the ends of the two electrodes 14. The access cover 20 is placed in position on top of the spark discharge chamber 12.

At this point, the entire apparatus is enclosed in a vacuum chamber and a vacuum is drawn down into the range of approximately 400-500 millimeters of mercury. As the vacuum is drawn, a supply of helium is bled into the vacuum chamber. Thus, the vacuum chamber contains a relative vacuum compared to the atmosphere and the atmosphere within the vacuum contains helium. The lower density of helium, combined with the reduced pressure, lowers the drag on both the carrier sheet 22 and the carrier particles.

The accelerated particle transformation process is initiated at this point. The voltage from the capacitors is electrically discharged to the electrodes 14. The voltages used in the present process have been in the range of 9-25 kV. The range of 9-10 kV is preferred. The voltage is discharged through the use

of appropriate electric switching described above. The force of the electric discharge initiates a spark which leaps the gap between the electrodes 14 and vaporizes the small droplet of water which was placed between the electrodes. The vaporization force creates a violent atmospheric shock wave within spark discharge chamber 12. The shock wave radiates outward from the electrodes in all directions. Because of the immovable sides of the chamber, the impact of the radiating shock wave upon the interior of the discharge chamber 12 is focused towards the carrier sheet 22, which is then propelled upward with great velocity. The upwardly traveling carrier sheet 22 accelerates upward at great force until it contacts the retaining screen 24. The displacement of the remaining atmosphere in the chamber with helium assists in the travel of the carrier sheet 22, since helium provides less drag on the flight of the carrier sheet as well as on the carrier particles themselves. At the retaining screen 24, the carrier sheet 22 impacts the retaining screen 24 and is retained. The nucleic acid-coated particles, in contrast, fly off of the carrier sheet and travel freely toward the target tissues. The small carrier particles then hit the living tissue on the target surface and proceed into the tissue cells.

C. Regeneration of Maize Plants

Plants must be created from the bombarded conditioned IES tissue. At either the cellular or plant level, the plants must be screened or selected to segregate the transformed plants from the nontransformed plants. In most particle-mediated plant transformation procedures done without selection, the nontransformed plants will be the large majority of the recovered plants. If a selection agent like hygromycin is used, the number of plants recovered is smaller but the relative proportions of transformant plants recovered is much higher. The selection regimen may not kill all of the non-transformed embryogenic cells,

but it has been found to be useful in enriching the pool of recovered plants so that germ line transformant plants are recovered with greater frequency.

After bombardment, the embryo tissue is fragile. It is considered advantageous to let the bombarded tissues remain on the culture medium for approximately four hours. If a gene encoding a selection marker has been incorporated into the cells, the regenerating plant must be placed on medium containing the selection agent at some point in the growth process. Many independent transformants have been obtained.

First, shoots must be induced from the transformed cells. Shoot induction methods are well-known in the art. Green and Phillips, Crop. Sci. 15:417-420 (1975) disclose one practical method for regenerating maize shoots from callus culture. Second, mature plants must be grown from the shoots. Kernels from mature transformed plants may then be analyzed for gene expression.

6. Gene Expression Assay Protocols

One will wish to determine the level of gene expression with the gene constructs of the present invention in many independent transformants. Preferably, the expression level is at least 1% of total soluble protein (TSP) in endosperm. More preferably, the level is at least 2.5% of total soluble endosperm protein. Even more preferably, the level is at least 5% of total soluble endosperm protein, and, most preferably, the level is at least 10% of this total soluble protein in endosperm.

Although we have expressed the protein connected to the rice glutelin-1 promoter in the maize kernel as a percent of endosperm protein, the product can also be expressed as a percentage of total soluble seed protein. It is more convenient to express the protein product as a percentage of total soluble endosperm protein because rice glutelin promoter is endosperm-specific. Thus, we expect to have maximal/optimal

expression in that tissue. Additionally, it is possibl and convenient to separate the maize endosperms (for ELISA assays) from the scutella (to obtain next generation plant and seeds). 64% of the total soluble protein and 97% of the BR96 (in a recombinant plant) is in seed endosperm. In corn seeds hull (pericarp and testa) contain ~4% of TSP and 2% of BR96 and germ has 32% of TSP and 0.4% of BR96. Most, if not all, of the BR96 antibody is concentrated in the endosperm. Therefore, BR96 as percent of endosperm TSP is approximately twice what it would be in the whole seeds.

To determine these seed and endosperm TSP levels, one needs to first extract protein from dry maize seeds or endosperm and then determine the level of gene product within that protein preparation. We detail below two preferable methods for extracting protein and performing an ELISA assay, but one of skill in the art would realize that there are many substitutions that can be made in these procedures. All that is really necessary is to determine the level of gene expression within a soluble protein extraction.

A. Protein Extraction from dry seed and endosperm

Surface sterilized seeds are placed on wet sterile filter paper overnight at room temperature to imbibe water. Holding seed firmly in one hand and using a scalpel, one then cuts around the perimeter of scutellum to remove the pericarp. The scutellum are removed by cutting deeper around it in the endosperm. The part of kernel remaining after removing scutellum is treated as endosperm because the remaining pericarp contains only 4% of total soluble protein and 2% of the total antibody.

Total soluble protein from the endosperm is essentially extracted the same way as the whole seeds except that the endosperms (because they are wet) are ground in pestle and mortar.

The following extraction buffer is useful for the

extraction of protein from dry seeds and endosperm:

Extraction Buffer (for 500 mls)

100 mls 0.5M NaPO4 (pH 7.0)	final conc. =	100 mM NaPO4
20 mls 0.5M EDTA (pH 8.0)		20 mM EDTA
10 mls 10% Triton X-100		0.2% Triton X-100
370 mls dH_2O		

(The buffer should be filter sterilized or made from sterile stocks.)

Individual maize seeds are crushed between layers of glassine weighing papers on an Arbor seed press. Ground material is poured into a 1.5 ml microcentrifuge tube. Approximately 1.5 ml extraction buffer is added. The mixture is vortexed on high for 5-10 seconds and then soaked at 4°C for at least 2 hours. The extractions are sonicated for 1.5-2 minutes. The tubes are centrifuged in a microcentrifuge set at highest speed for 20 minutes at 4°C to remove cell debris. All of the supernatant is transferred to fresh tube. A set of protein standards is prepared using 1 ug/ul human IgG and unknowns using Coomassie Plus Protein Assay kit from Pierce. Absorbance is read at 595 nM. Unknowns are compared to IgG standard curve to determine total protein in seed extract.

Whole maize seed should typically have 8-12 ug total soluble protein (TSP)/ul extract. Endosperm only should have about 5-6 ug TSP/ul extract.

B. ELISA Assay

5 ul of capture antibody (goat anti-human, gamma-specific) are combined with 5.5 mls of Capture Buffer (50 mM Na_2CO_3 pH 9.6). 50 ul are loaded per well in a microtiter plate to coat plate and stored O/N at 4°C.

Wells are washed 3X with PBS + 0.25% Tween20 (PBST), using plate washer. Blocking Solution (0.5 g Carnation Milk Powder in 25 mls PBST) is prepared and 200 ul is loaded per well. The plate is incubated at RT for 1 hour. Wells are washed 3X with PBST using plate washer.

The dilution plate is prepared as follows: load samples into head wells (vertical row), make 3 or more serial 1:2 dilutions (in PBS) across plate using the 8-channel pipettor. Start with 200 ul (total volume) in head well and 100 ul in each dilution well. Pipet 100 ul from head well into first dilution well and mix. Remove 100 ul from first dilution well and mix with the next well, etc.

For a standard curve, use 2 ng of protein standard/50 ul in head well (Pipet 4 ul of 2 ng/ul BR96 into 196 ul PBS in headwell of dilution plate to give 2 ng/50 ul on ELISA plate). (For example, in the examples below, we used two ng of BR96 standard per 50 ul in the headwells.)

50 ul of each dilution is loaded onto ELISA plate. Incubate at 37°C for 1 hour. Wells are washed 3X with PBST using plate washer.

5 ul of detection antibody (goat anti-human, kappa-specific--HRP conjugate) is prepared in 5.5 mls PBST. 50 ul is loaded per well and incubated at 37°C for 1 hour. Wells are washed 3X with PBST using plate washer.

Substrate solution is prepared according to BioRad kit directions (9 mls TMB peroxidase solution A + 1 ml solution B), loaded 100 ul per well and incubated at RT for exactly 10 minutes. The reaction is stopped by loading 100 ul of 1M H_2SO_4 per well. Absorbance is read at 450 nM.

Unknown samples are compared to protein standard curve to get ng in the protein extract. Divide ng protein extract by ug total soluble protein (TSP)/ul extract to get % protein/TSP.

Examples

1. Comparison of Various Promoters

We wanted to investigate various promoters and their ability to drive gene expression in corn kernels. To that end, we selected the promoters described below at Table 1 and Table 2 and created gene constructs

where the promoters were operably connected to the BR96 heavy chain and light chain genes.

For two promoters (rice glutelin-1 and 27-kD-zein), the heavy chain and light chain were on two different plasmids. For all other plasmids, the heavy chain and light chain genes were on the same plasmid. If two plasmids were necessary, the plasmids were transformed together. For a positive ELISA reaction, both the light chain and heavy chain have to be expressed. If light and heavy chains are expressed at different levels, the ELISA assay would only indicate expression indicative of the lower expression level.

We chose this monoclonal antibody BR96 as an example of an antibody that would be usefully commercially produced. Monoclonal antibody BR96 binds selectively to carcinomas of the colon, breast, ovary and lung. This monoclonal antibody is described in Hellstrom, et al., Cancer Research 50:2183-2190, 1990, and Trail, et al., Science 261:212-215, 1993.

The gene constructs containing both the light and heavy chain of BR96 and the rice glutelin-1 promoter are described above and at Figs. 4 and 5.

These gene constructs were transformed, as described above and below at Example 2, into maize embryos and transgenic plants were ultimately derived from the bombarded embryos. The plants were transformed by using WRG5084 and WRG5086 plasmid DNA. For preparing the gold bead preparation, for bombarding the conditioned immature embryo scutella, the DNA were mixed in equal proportions (for co-transformations). As described above, in both plasmids, the heavy chain (HC) or light chain (LC) of BR96 is driven by the rice glutelin promoter. "ITE" designates independent transformation events. This designation is based on which callus the plants originated from. Table 1 tabulates the ELISA values (as percentage of total soluble endosperm protein) in the R_1 progeny of these transgenic plants.

TABLE 1

Effect of Promoter on Monoclonal Antibody BR96 Expression (heavy chain and light chain) in Endosperm of R1 Corn Seeds

Plasmid #	Promoter	# of Independent Transgenic Plants	R1 Seed Endosperm ELISA* MEAN	RANGE	# of Endosperm and/or Seeds Assayed
WRG2841	35S	4	0.29	0.03 to 0.71	67
WRG2926	35S-ADH	5	0	0	68**
WRG2981	Ubi-1	9	0.61	0.10 to 0.98	8 & 38**
WRG5003/5005	maize 27kD-ADH	26	0.07	0.01 to 0.39	227
WRG5084/5086	Rice glutelin-1	9	3.67	0.15 to 10.1	122
WRG5117	Soy-7S	11	0.01	0.01 to 0.02	64**
WRG5193	35S/ppdK	4	0.02	0.02	60**
WRG5216	maize glob-3	10	0.08	0.01 to 0.17	17 & 157**
WRG5217	35S-glob-3	5	0.07	0.0 to 0.26	4 & 53**

*ELISA values were expressed as percent of total soluble protein. ELISA values of R1 endosperms only. Scutella were used to obtain R2 plants.
**ELISA assays performed on whole seed protein. ELISA values for whole seed protein were similar to endosperm values in transformants with plasmids WRG5117, WRG5216 and WRG5217.

As Table 1 indicates, gene constructs containing rice glutelin-1 promoter express the foreign mammalian gene, in this case antibody BR96 heavy chain and light chain, to a level of between .15 to 10.1% of the total soluble protein in endosperms. This level stands in sharp contrast to the other promoters evaluated, which never expressed BR96 gene at a level of more than approximately 1% of the TSP.

Additionally, another rice glutelin promoter, GT3, was examined in transgenic rice. 850 bp of the GT3 promoter (Leisy, et al., Plant Mol. Biol. 14:41-50, 1989) was fused to the GUS gene. This construct was used to transform Gulfmont rice. Transformants were negative for GUS expression in rice seed (Cooley, et al., Theoretical and Applied Genetics, 90:97-104, 1995).

Table 2 below describes the various promoters used in the Table 1 analysis.

TABLE 2

Promoter	Size (kb)	Plasmid(s)	Reference
Rice Glutelin 1 -4861 to +27	4.9	WRG5084 BR96 HC WRG5086 BR96 LC WRG4564 GUS WRG5194 MC451	Zheng, et al. (1993) The Plant Journal 4, 357-366; SEQ ID NO:1
CaMV 35S -440 TO +1	0.44	WRG2119 GUS WRG2841 BR96 HC+LC	Gardner, et al. (1981) Nucleic Acids Res 9:2871-2888
CaMV 35S ADH intron -440 to +1 of 35S fused to +120 to +672 of ADH intron	0.96	WRG5154 GUS WRG2121 GUS WRG2926 BR96 HC+LC	Gardner, et al. (1981) Nucleic Acids Res 9:2871-2888 Callis, et al. (1988) Genes and Development 1:1183-1200
27kD Zein* -1140 to +60	1.14	WRG2952 GUS	Das, et al. (1990) Genomics 11:849-856
27kD Zein ADH intron** -1140 to +60 of 27kD fused to +120 to +672 of ADH intron	1.69	WRG5003 BR96 HC WRG5005 BR96 LC WRG2973 GUS	Das, et al. (1990) Genomics 11:849-856 Callis, et al. (1988) Genes and Development 1:1183-1200
Maize Globulin -1314 to +4	1.32	WRG5216 BR96 HC+LC WRG5149 GUS	Belanger & Kriz (1991) Genetics 129:863-872
Maize Ubiquitin 1*** -900 to +1090	1.99	WRG2943 GUS WRG2980 BR96 HC+LC	Christensen, et al. (1992) Plant Mol Biol 18:675-689
Soy conglycinin (7S) -900 to +7	0.9	WRG5117 BR96 HC+LC WRG2728 GUS	Chen, et al. (1986) Proc. Natl Acad Sci USA 83:8560-8564
CaMV 35S -430 to - 40 fused to Maize PPDK -51 to +139	0.44	WRG5193 BR96 HC+LC	Jen Sheen (1991) The Plant Cell 3:225-245

*-60 to - 70 was replaced with pUC polylinker and Nco1 linker

**a 40 bp linker/leader between intron and first AUG is from CMC1208nco

***promoter and 1st exon w/ intron fused to Nco1

2. BR96 Expression in R_1 and R_2 Maize Kernels of Independent Transformation Events.

Table 3 below tabulates the individual rice glutelin-1 promoter transformants that were summarized in Table 1 above. In the Table 3 experiments, we assayed the expression levels of BR96 as percentage of total soluble protein in endosperms of transgenic R_1 maize kernels. Endosperms were separated from scutella to run the ELISA assays, and scutella were used to grow R_1 plants. The resulting R_2 seeds from R_1 plants were assayed to determine the transgene transmission and stability of level of expression. The results are presented in Table 3. High BR96 expression levels were obtained for many different transformants in both R1 and R2 generations suggesting the stability of transgene expression over generations. In general, for rice glutelin-1 promoter, the endosperm ELISA values for BR96 were about twice that of whole kernel ELISA values.

TABLE 3
Rice Glutelin-1 Promoter Provides High Expression Levels of BR96 in Endosperms and Whole Corn Seeds

Expt #	R_0 plant #	R_1 Seed Endosperm ELISA* mean	range	ELISA positive R_1 seeds/total	R_2 Whole Seed ELISA** mean	range	ELISA positive R_2 seeds/total
C982	12A1	2.9		1/15	0	0	0/8
C982	12A2	5.1	3.5-7.6	2/13	0	0	0/12
C983	A634xC983 6B1	3.3		1/10	4.0	1.8 to 6.1	11/18
C983	8A1	2.7	1.2-4.1	10/20	2.3	0.01 to 6.9	105/130
C983	A634xC983 8A1	5.5	4.0-6.9	2/5	1.7	1.1 to 2.6	6/15
C984	16A1	4.5	1.1-10.1	34/50	2.6	0.7 to 7.4	172/214
C984	16A2	2.5	1.4-3.5	6/10			
C984	16A3	3.5	1.9-5.3	10/15	1.7	0.5 to 3.7	28/38
C984	16A4	3.0	1.8-4.7	9/10	3.9	2.8 to 5.8	4/5
C984	16A5	3.0	1.8-4.9	7/10	1.2	0.8 to 1.8	11/15
C984	16A6	3.8	2.0-5.6	13/20	2.7	0.5 to 8.1	67/70
C984	A634x984 16A1	4.2	3.8-4.7	4/11	1.4	0.9 to 3.4	32/53
C984	A634x984 8A3	0.4	0.2-0.6	4/10	0.1	0.05 to 0.1	12/35
C985	9A1	5.0	1.0-8.9	24/37	3.4	1.2 to 6.8	36/48

*ELISA values of R_1 endosperms only. Scutella were used to grow plants to obtain the next generation.
**ELISA values for whole R_2 seeds. Only ELISA positive seeds were used to calculate the means and range of values.

As Table 3 indicates, the mean percentage of BR96 in TSP for different transformants was 0.4 to 5.5% and ranges from 0.4 to 10.1%.

3. NeoRx451 Expression in Maize Kernels

We have assayed another antibody gene to demonstrate that the increased expression due to the rice glutelin-1 promoter would work with other mammalian genes. Table 4 (below) describes an experiment in which the rice glutelin-1 promoter was placed upstream of the NeoRx451 heavy chain and light chain genes. The transforming plasmids containing the NeoRx451 light and heavy chains respectively, WRG5243 and WRG5236, are illustrated in Figs. 8 and 7. The heavy chain of this antibody is naturally glycosylated. A version of the heavy chain has been modified to remove the glycon addition site. Both the glycosylated (WRG5245 Fig. 9) and the nonglycosylated (WRG5236 Fig. 7) versions of the heavy chain have been used in these experiments.

Seven different transformation events produced transformants containing the nonglycosylated antibody from WRG5243 and WRG5236. The R1 seed of these transformants was analyzed with the following results: The NeoRx451 monoclonal antibody was expressed as 0.4 to 3.5% of endosperm total soluble protein. When whole R_2 seeds were measured, mean NeoRx451 ELISA values were 5.0% and ranged from 1.6 to 12.8% of total soluble seed protein.

For producing the glycosylated version of NeoRx 451, the transforming plasmids containing the NeoRx-light and heavy chains (glycosylated) respectively, WRG5243 and WRG5245, are illustrated in Figs. 7 and 9.

Ten independent transformants were produced. The R_1 seed of these transformants was analyzed with the following results: The NeoRx451 monoclonal antibody was expressed as 11.0 to 25.7% of endosperm total soluble protein and 0.3 to 15.0% of whole seed TSP (Table 4). Mean whole seed ELISA values, for different

transformants, varied from 0.3 to 9.8%, whereas mean ELISA values for the endosperm was 16.7% (C2015-7A1 transformant). These are quite high expression levels for transgenic protein.

TABLE 4

Rice Glutelin-1 Promoter Provides High Expression Levels of Glycosylated and Non-Glycosylated Forms Of Monoclonal Antibody NeoRx451 in Endosperms and Whole Corn Seeds

Plasmid #	Promoter	Genes	Exp. #	R_0 Plant #	R_1 Seed ELISA Values mean	range	# of ELISA Positive R_1 Seeds/Total		ELISA R_2 Seeds mean	range	ELISA Positive R_2 Seeds/Total
WRG5236/5243	Rice Glutelin-1	NeoRx451 HC&LC	1076	5A1	1.6	0.4-3.5	29/35	5.4	1.6-12.8		15/16
WRG5236/5243	Rice Glutelin-1	NeoRx451 HC&LC	1076	5A2	1.2	0.6-2.6	16/25	4.7	2.5-6.9		5/9
WRG5236/5243	Rice Glutelin-1	NeoRx451 HC&LC	1079	5A1	0.4	0.1-0.8*	8/10				
WRG5236/5243	Rice Glutelin-1	NeoRx451 HC&LC	1094	5A1xA634	2.3	1.8-2.8	3/5				
WRG5236/5243	Rice Glutelin-1	NeoRx451 HC&LC	1094	A634x5A1	1.1	0.8-1.4	2/5				
WRG5245/5243	Rice Glutelin-1	NeoRx451 HC&LC	2019	A634x2019-4A1	2.5	2.3-2.6	2/5				
WRG5245-5243	Rice Glutelin-1	NeoRx451 HC&LC	2019	A634x2019-8A3	0.7	0.5-0.8	2/5				
WRG5245-5243	Rice Glutelin-1	NeoRx451 HC&LC	2010	A634x2010-1A2	0.3	0.3	1/5				
WRG5245/5243	Rice Glutelin-1	NeoRx451 HC&LC	2015	7A1	9.8	7.3-15.0	8/10				
				7A1	16.7*	11.0-25.7*	7/10				

*ELISA values of R_1 Endosperms only. Scutella were used to grow plants to obtain the next generation. Only ELISA-positive kernels were used to calculate the means and range of values.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: MONOCOT SEED GENE EXPRESSION SYSTEM

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4891 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTACCCCTC GCTTCACTCC AAGCTCCACG GCGGCGGCTT CTTGCCCCTG CAATCACTGG 60

CCAGCCTGCC CAAGAGGATA AAAGTGAGAG AAAGAGAGGA GGAAGGGAGA TGAGGGGAAA 120

GAGGGAGGTG ATGACATGGA TTACTGATAT GTAGGGTTCA CGTGGGTTCC ACGCTGACTC 180

AGCCGCCACG TCGGATAAAA CCGGGATCAA AGCTACCGAA TGACCTAAAG TGAACAGTTT 240

TGTAAATTGA GGGATGTCAT GTATCCGGTT TTGTGGTTGA TGGACGATTT TGTAACTCGA 300

TGACAAATTG AGCGACCTGC GGTGTACTTT TTCCTTCCGC CCTGTGTGGA GGCCCAAACA 360

TTCAGCCCAT TCCCAACCTG GCACTGACAT GCGGGCCATT CCAAAGCCTT GCACAGTTTC 420

ACCTCTCACC CGCGCCTCCG CTTCCTCCCG CCTCCCCAAA CGATGCCGCC TCCGCCTCCG 480

TCTTCCCGTC TCCTCGCCCT CCTCTCCGCG CGCCGCCCGC CGCCCCCGCT CCGCCGCCTC 540

CTCCAGATCC ACGCCCACCT CCTCGCCGCC GGCCTCCTTC AAGACTTCTC CTCCCTCCTC 600

GCCGCCGCCT ACGCGCTCTC CACCACCGCC ACCGCCACGG ACGCCCGCAC CTCGCCGCCC 660

TCCCCGCTCC GCCACGCGCT CGCGCTCCTC TCCTCGCTCC CGGCCTCCGC CTACAACGCC 720

GCCATCCGAG CACTCTCCCT CTCCGACGAC GGCGACCGCC ATGGCCACGG CGTCGTCCGC 780

CGCTGCCTCC CGCTCTACCG CGCCCTCCTC CGCTCCGGGA CCGCGCGCCC CGACCACCTC 840

ACGTTCCCGT TCCTGCTCAA GGCCTGCGCG CGCCTGCGGG AGTGGGGATA CGGCGACGCG 900

GCCCTCGCGC ACGTCCTCCG CCTCGGCCTC GACTCCGACG TCTTCGTGGT GAACGCGGCC 960

ACGCACTTCC TATCGATCCG CGGGCCCATG GAGGACGCAC GCAGGCTGTT CGACCGAAGT 1020

CCTGTGAGGG ACTTGGTGTC GTGGAACACG CTGATCGGAG GGTACGTGCG GCGGGGGAAC 1080

CCAGCGGAGG CGCTGGAGCT GTTCTGGAGG ATGGTGGCAG AGGATGCAGT GGTGAGGCCT 1140

GATGAGGTCA CGATGATCGC GGCTGTGTCG GGGTGTGGGC AGATGCGTGA CCTGGAGCTT 1200

GGGAGGCGGC TTCATGGGTT CGTGGATAGT GACGGAGTGA GTTGCACTGT GAGGCTGATG 1260

AATGCGCTGA TGGATATGTA CATCAAGTGT GGCAGTTTAG AGATGGCAAA GTCTGTGTTC 1320

GAGAGGATCG AGCACAGGAC AGTTGTCTCT TGGACGACGA TGATCGTGGG GTTTGCCAAG 1380

TTCGGATTGA TGGACGATGC ACGTAAAGTG TTTGATGAGA TGCCTGAAAG GGATGTGTTC 1440

CCATGGAATG CACTCATGAC CGGTTATGTG CAGTGTAAGC AGTGCAAGGA GGCCCTTTCC 1500

TTGTTTCATG AGATGCAGGA AGCAAGTGTG GTGCCTGATG AGATCACAAT GGTCAATCTT 1560

CTAACTGCTT GTTCGCAGCT CGGAGCATTA GAAATGGGGA TGTGGGTTCA CCGGTACATT 1620

GAGAAACATC GCCTTGTATT TAGTGTTGCG CTTGGCACAT CTCTCATTGA CATGTACGCT 1680
AAGTGTGGAA ACATTGAGAA AGCTATCCAC ATTTTCAAAG AAATTCCCGA GAAAAATGCA 1740
CTCACATGGA CAGCAATGAT ATGTGGTCTA GCAAATCATG GACATGCCAA TGAGGCCATA 1800
GAGCACTTCC GGACAATGAT AGAGCTTGGA CAGAAGCCAG ATGAGATTAC GTTTATAGGT 1860
GTTCTTTCAG CATGCTGTCA TGCTGGTTTG GTGAAAGAAG GTCGGGAATT TTTCTCTCTG 1920
ATGGAGACAA AATATCATCT TGAGAGGAAA ATGAAACATT ATTCATGTAT GATAGACTTA 1980
CTAGGCAGGG CAGGCCATTT AGACGAAGCA GAGCAGCTAG TAAACACTAT GCCTATGGAA 2040
CCTGATGCAG TAGTTTGGGG TGCTATCTTC TTTGCTTGTA GGATGCAAGG TAATATCTCT 2100
CTTGGAGAAA AGGCAGCAAT GAAATTGGTA GAAATTGATC CTAGTGATAG TGGAATCTAT 2160
GTGCTACTGG CTAATATGTA TGCAGAAGCG AACATGAGGA AGAAGGCTGA CAAAGTCAGG 2220
GCTATGATGA GACATTTGGG AGTGGAGAAA GTTCCTGGGT GTAGCTGCAT TGAGTTGAAT 2280
GGTGTGGTTC ATGAATTTAT CGTGAAGGAC AAGTCACATA TGGATAGTCA TGCTATTTAT 2340
GACTGCTTGC ATGAGATCAC CCTACAAATA AAGCATACTG CAGATTTGCT TAGCATTTCT 2400
GCGGCTGGTG CGGTGTAGTG TTCTGTTGGC TGGAACAGCT GGCTGAGCTG TGCAAGATGA 2460
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GGTTTGCCAG GCCATGTTCT GAGAAGGGTA TACTTCATGT TGATTACTAT CTGAGGCATT 2580
CCCCGAGAAT TTTCTGGTCG TTCTTTTGCA GCTTGATGTC AATGGAAACA ATATGTTCCA 2640
CTACATATTG CAAAGTTCTT GTATGCTCTT TACTCAACCC TCACGTGCGG AGCACTTCCT 2700
GGGTAAGTGT GGTTCTCATG CTCTGTTTTG CCTCCTCCAT TTCTCCTCCG TTGCATTTAA 2760
AGTCACATCC CCCTCCTCAG GTTTTCTCCA TTAGCTCTCT GTAGTCCTTG CTGTACTCTC 2820
CTTGGTATTC CATGCTGTCC TACTACTTGC TTCATCCCCT TCTACATTTT GTTCTGGTTT 2880
TTGGCCTGCA TTTCGGATCA TGATGTATGT GATTTCCAAT CTGCTGCAAT ATGAATGGAG 2940
ACTCTGTGCT AACCATCAAC AACATGAAAT GCTTATGAGG CCTTTGCTGA GCAGCCAATC 3000
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CTTAACATTT AGATGCAAGA GCCTTTATCT CACTATAAAT GCACGATGAT TTCTCATTGT 4860

TTCTCACAAA AAGCATTCAG TTCATTAG 4888

Claims

We claim:

1. A gene construct comprising:
a rice glutelin-1 promoter operably connected to a foreign non-plant gene.

2. The gene construct of claim 1 wherein the promoter is SEQ ID NO:1.

3. The gene construct of claim 1 wherein the promoter comprises nucleotides 3021 to 4888 of SEQ ID NO:1.

4. The gene construct of claim 1 wherein the gene construct is part of a plasmid or viral vector.

5. The gene construct of claim 1 wherein the foreign gene is mammalian.

6. The gene construct of claim 1 wherein the foreign gene is an antibody or antibody fragment.

7. A monocot plant containing the gene construct of claim 1.

8. The plant of claim 7 wherein the plant is a maize plant.

9. A monocot plant cell containing the gene construct of claim 1.

10. The cell of claim 9 wherein the cell is a maize cell.

11. A monocot seed containing a gene construct comprising a rice glutelin-1 promoter operably connected to a foreign non-plant gene, wherein the promoter and gene are not natively connected.

12. The seed of claim 11 wherein the seed is a maize kernel.

13. The seed of claim 11, wherein a product encoded by the foreign gene is expressed at a level of at least 1% of total soluble endosperm protein.

14. The seed of claim 13 wherein the level is at least 2.5% of total soluble endosperm protein.

15. The seed of claim 14 wherein the level is at least 5% of total soluble endosperm protein.

16. The seed of claim 15 wherein the level is at least 10% of total soluble endosperm protein.

17. The seed of claim 11 wherein the non-plant gene is an antibody.

18. The seed of claim 12 wherein the non-plant gene is an antibody.

19. A method of expressing a non-plant gene in a monocot cell, comprising the steps of

a. creating a gene construct comprising a rice glutelin-1 promoter operably connected to a foreign non-plant gene,

b. creating a transgenic monocot cell comprising the gene construct, and

c. allowing expression of the non-plant gene in the monocot cell.

20. The method of claim 19 wherein the monocot is maize.

21. A method of expressing a non-plant gene in a monocot plant, comprising the steps of

a. creating a gene construct comprising a rice glutelin-1 promoter operably connected to a foreign non-plant gene,

b. creating a transgenic monocot plant comprising the gene construct, and

c. allowing expression of the non-plant gene in the monocot plant.

22. The method of claim 21 wherein the monocot is maize.

23. A method of expressing a non-plant gene in a monocot seed, comprising the steps of

a. creating a gene construct comprising a rice glutelin-1 promoter operably connected to a foreign non-plant gene,

b. creating a transgenic monocot seed comprising the gene construct, and

c. allowing expression of the non-plant gene in the monocot seed.

24. The method of claim 23 wherein the monocot is maize.

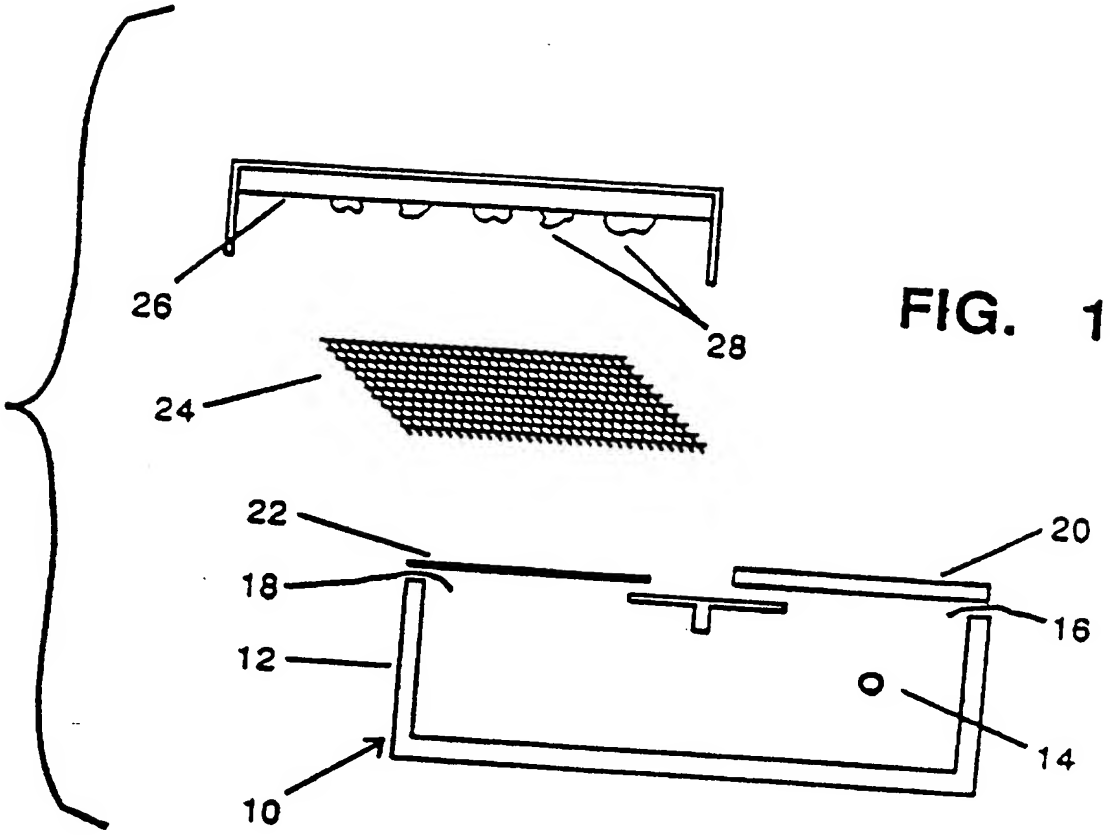


FIG. 1

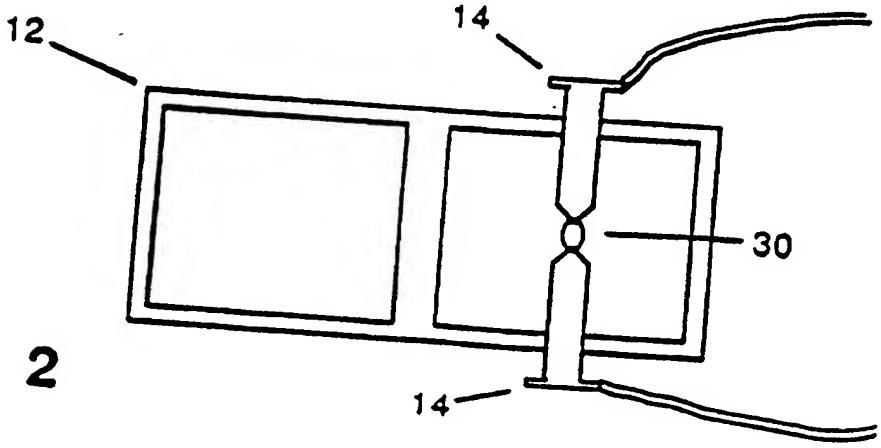


FIG. 2

FIG. 3

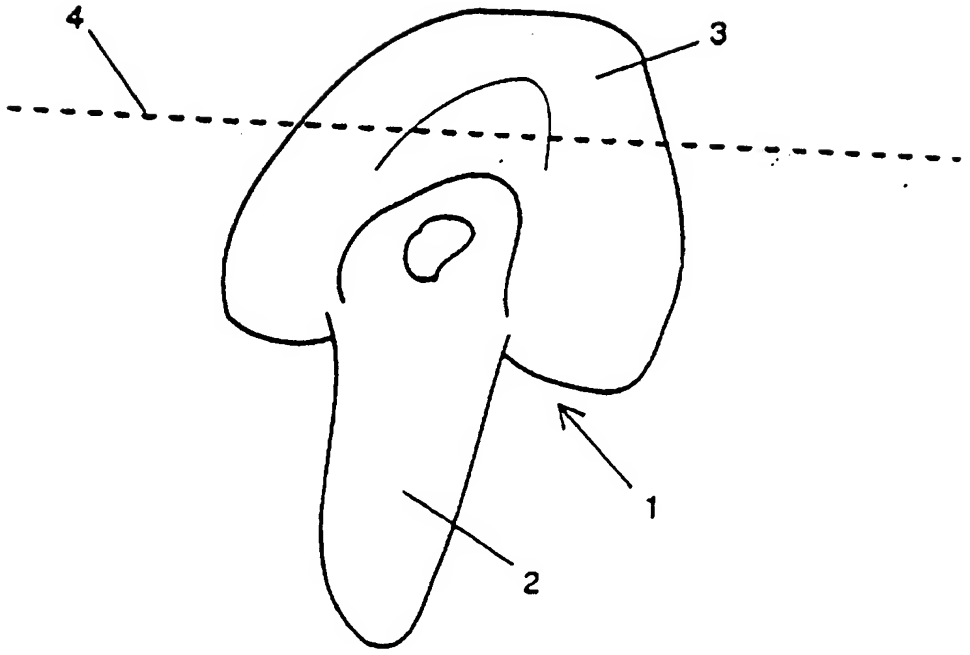


FIG. 4

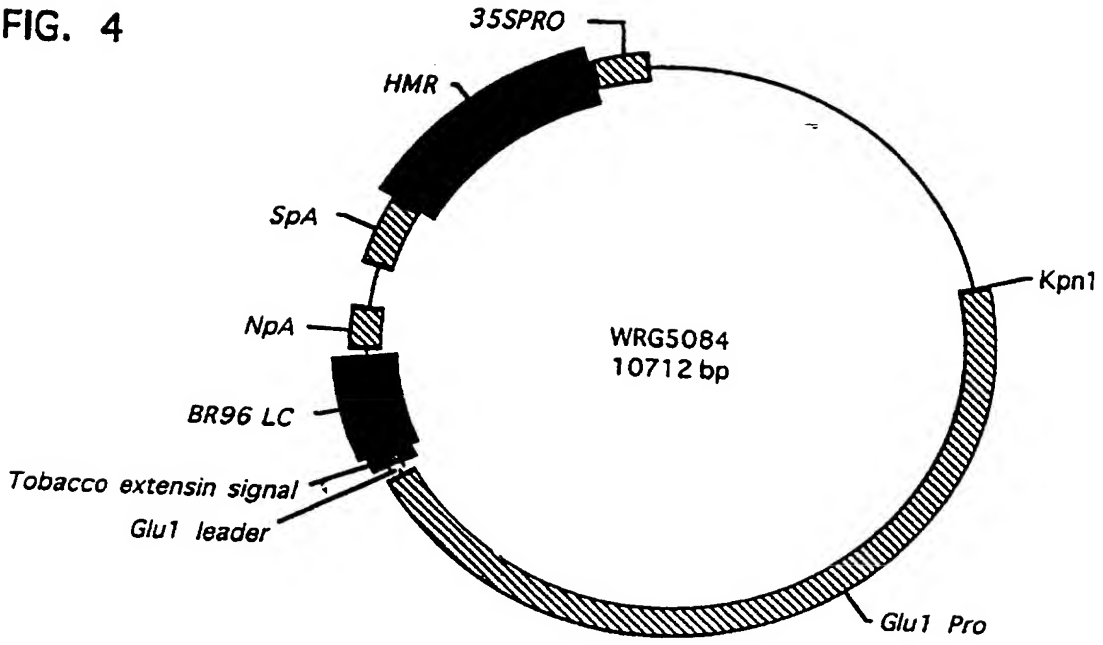
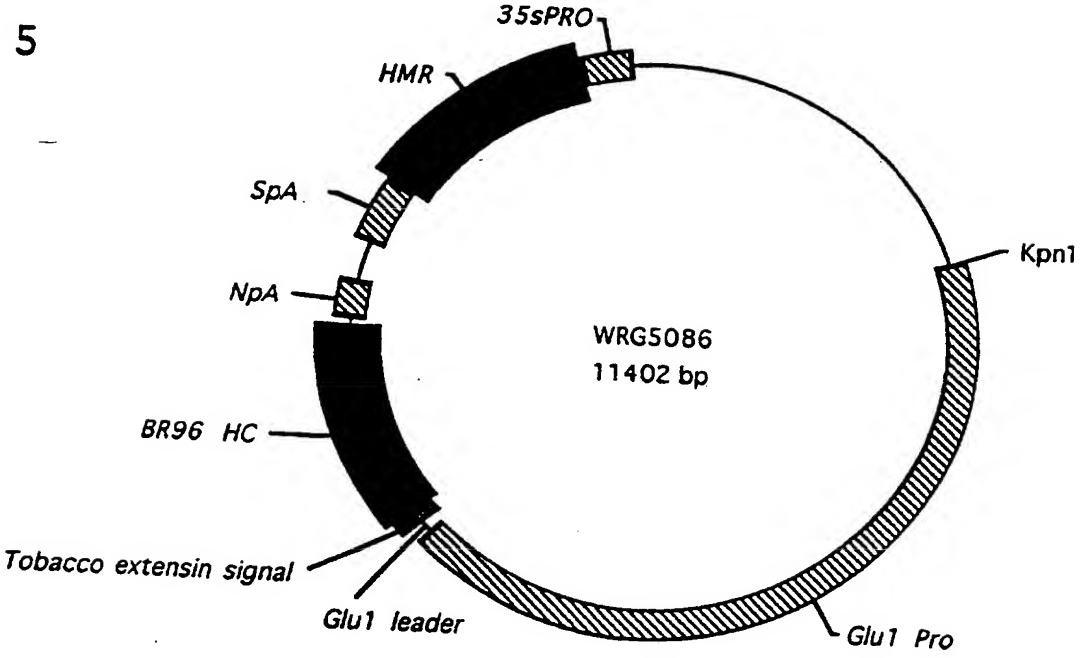
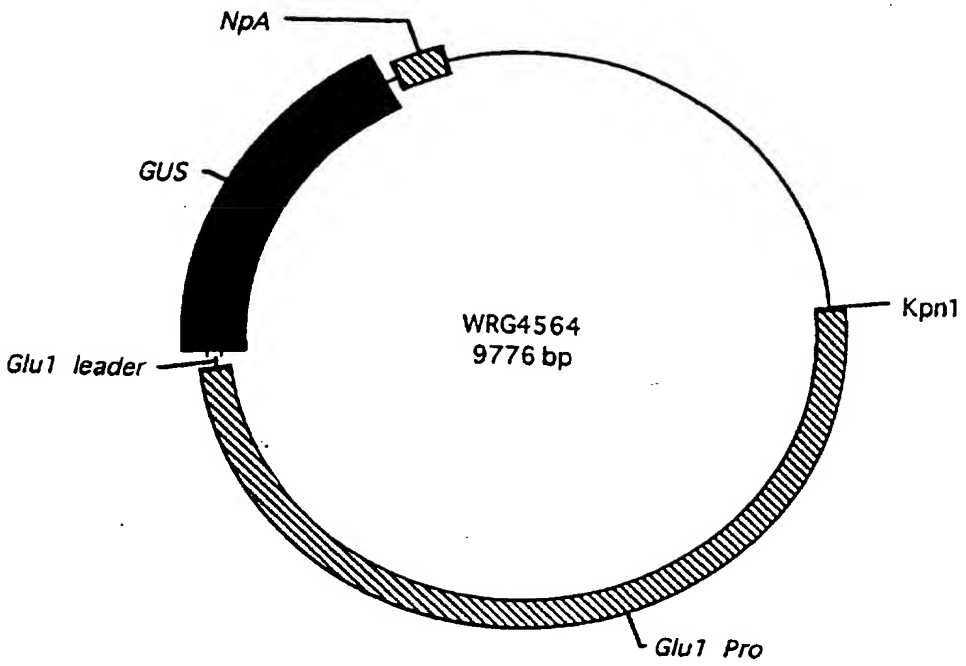


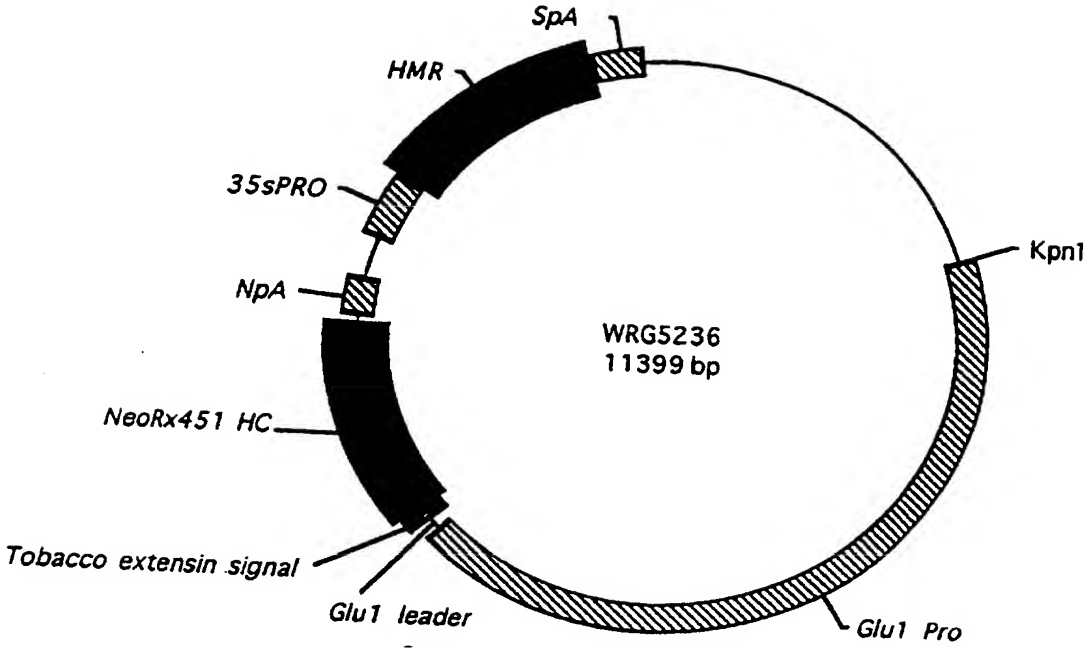
FIG. 5





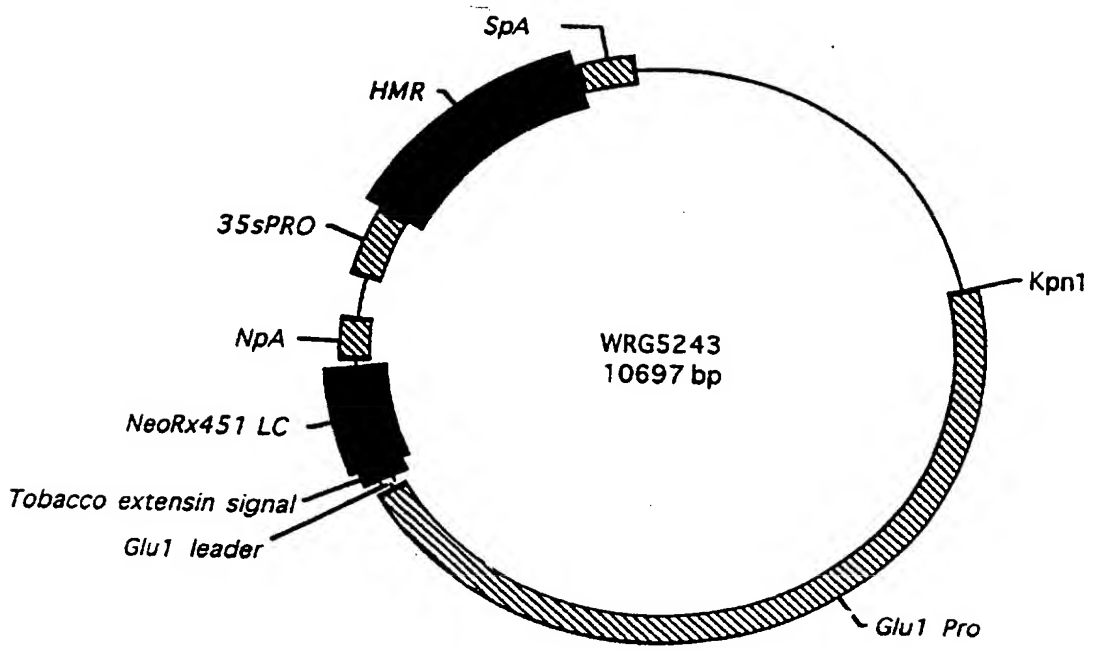
Glu1 promoter= bp 2260 → 7121

FIG. 6



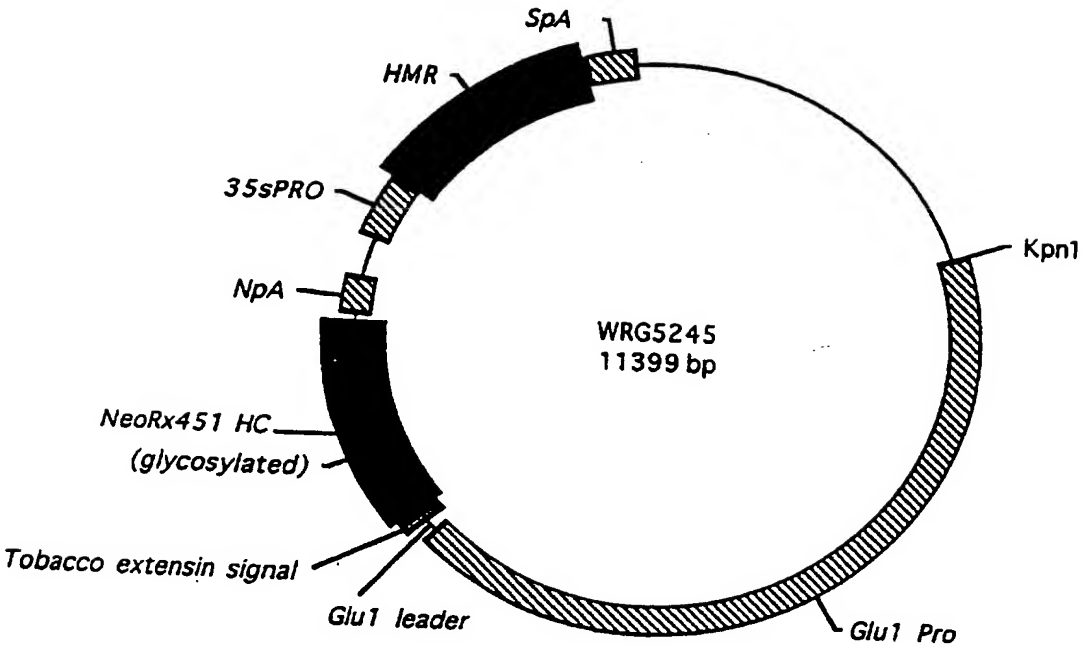
Glu1 promoter= bp 2260 → 7121

FIG. 7



Glu1 promoter= bp 2260 → 7121

FIG. 8



Glu1 promoter= bp 2260 → 7121

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
[redacted]US97/15340

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00, 15/09, 15/12, 15/29, 15/64, 15/82; A01H 4/00, 5/00
US CL :800/205; 435/69.1, 172.3, 320.1, 419; 536/23.5, 23.6, 24.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/205; 435/69.1, 172.3, 320.1, 419; 536/23.5, 23.6, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CABA, CAPLUS, MEDLINE, BIOSIS
search terms: transgenic, recombinant, transformed, rice, glutelin, mammal, mammalian

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEISY et al. Expression of a rice glutelin promoter in transgenic tobacco. Plant Molecular Biology. 1989, Vol. 14, pages 41-50, see entire document.	1-24
Y	TAKAIWA et al. Analysis of th 5' flanking region responsible for the endosperm-specific expression of a rice glutelin chimeric gene in transgenic tobacco. Plant Molecular Biology. 1991, Vol. 16, pages 49-58, see entire document.	1-24
Y	US 4,956,282 A (GOODMAN et al) 11 September 1990, columns 1-10.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
14 OCTOBER 1997

Date of mailing of the international search report
30 OCT 1997

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Form PCT/ISA/210 (second sheet)(July 1992)★